5. Summary of Data Reported and Evaluation

5.1 Exposure data

The liver flukes, *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*, are biologically similar, food-borne trematodes which chronically infect the bile ducts and, more rarely, the pancreatic duct and gall-bladder of human beings and other mammals. Infection is acquired by eating raw or undercooked freshwater fish which contain the infective stage (metacercaria) of flukes. Immature flukes migrate up through the ampulla of Vater to the biliary tree, mature in the small intrahepatic ducts and produce eggs, which are passed in the faeces. If the eggs reach a water body and are consumed by an appropriate species of snail, they hatch and undergo asexual multiplication to produce free-swimming larvae, which can penetrate freshwater fish and become encysted metacercariae.

Liver fluke infections are best detected by identification of eggs in the faeces. In light infections and severe disease with obstruction, eggs may not be found. There is a close quantitative relationship between the number of eggs per gram of faeces and the number of adult worms. Immunodiagnostic techniques cannot be used reliably to detect active infections.

Nine million people are infected with *O. viverrini*, which is common in North-east Thailand, at least one-third of the population being infected, and in North Thailand and Laos. *O. felineus* affects 1.5 million people, mainly in the central part of the Russian Federation. An estimated 7 million people are infected with *C. sinensis* in the Republic of Korea, southern China, Hong Kong, Macao and Viet Nam. The distribution of human infection is determined primarily by the distribution of the habit of eating raw freshwater fish; heterogeneity within endemic areas is probably due to environmental factors and control. Infection generally occurs during the first decade of life, often with a similar pattern in men and women, although men may be more frequently and heavily infected than women.

Most liver fluke infections lead to local inflammation, and they are associated with specific clinical signs and symptoms in 5–10% of infected people. The intensity of infection is

correlated with hepatobiliary tract abnormalities visualized by ultrasound. Biliary and gall-bladder stones are commoner among individuals heavily infected with *Clonorchis* than among those infected with the other liver flukes. Treatment with praziquantel is highly effective and also leads to reversal of biliary tract abnormalities. Control of infection has been achieved in some areas by a combination of chemotherapy, health education and improved sanitation.

5.2 Human carcinogenicity data

Opisthorchis viverrini

Within Thailand, the highest proportional incidence rate of cholangiocarcinoma is observed in the north-east region of the country where the prevalence of infection with *O. viverrini* is also highest. In this region, the incidence of cholangiocarcinoma is about 40 times the highest incidence outside Thailand. A formal analysis across five regions of the country showed a strong correlation between proportional incidence of cholangiocarcinoma and estimated average titres of antibodies to *O. viverrini* and, to a lesser degree, faecal egg count. Correlations with proportional incidence rates of hepatocellular carcinoma were much weaker.

Many cases of liver cancer arising in patients with *O. viverrini* infection have been reported from Thailand. In most regions of the world, cholangiocarcinoma is a very rare tumour. In areas where *O. viverrini* is endemic, however, the numbers of cases of cholangiocarcinoma generally outnumber those of hepatocellular carcinoma.

Three cross-sectional or case–control studies of the association between infection with *O. viverrini* and cancer of the liver have been reported from Thailand. In the earliest and smallest of these studies, the estimated relative risks for cholangiocarcinoma and hepatocellular carcinoma in association with the presence of *O. viverrini* eggs in faeces were each 1.3. In the second study, the estimated relative risk for the association between cholangiocarcinoma and the presence of *O. viverrini* antibodies in serum was 5.0, which was significant. The association was not explained by possible confounding with hepatitis B virus infection or estimated recent intake of aflatoxins. The estimated relative risk for the association with hepatocellular carcinoma was 1.7 (not significant). In the third study, based on 15 cases of cholangiocarcinoma, estimated relative risks of 1.7, 3.2 and 14.1 were calculated for categories of faecal excretion of increasing numbers of *O. viverrini* eggs. This trend was highly significant.

Opisthorchis felineus

The incidence of liver cancer was observed to be correlated with the prevalence of infection with *O. felineus* across four areas in the T'umen' region of north-west Siberia. Cases of both cholangiocarcinoma and hepatocellular carcinoma have been reported in people infected with *O. felineus*.

Clonorchis sinensis

Cases of cancer of the liver in association with infection with *C. sinensis* have been reported from China, Hong Kong, the Republic of Korea and Japan and in immigrants to North America from China and Laos.

Two case–control studies of the relationship between *C. sinensis* infection and liver cancer, with partially overlapping case series, have been carried out in the Republic of Korea. Significantly increased estimated relative risks of 6.5 and 6.0 were seen for an association with cholangiocarcinoma, but no significant association was seen with the occurrence of hepatocellular carcinoma. In a third case–control study, in Hong Kong, the estimated relative risk for cholangiocarcinoma, after adjustment for age and sex, was 3.1, while that for hepatocellular carcinoma was 0.7.

5.3 Animal carcinogenicity data

Infection with *O. viverrini* alone was evaluated in hamsters in several studies that were not designed specifically as long-term carcinogenicity studies. Two cholangiocarcinomas were found in one of these studies. In several studies in hamsters infected with *O. viverrini* and treated with various carcinogenic *N*-nitrosamines, induction of cholangiocarcinomas and of hepatocellular nodules was enhanced.

No study of the carcinogenicity of *O. felineus* was available.

Infection with *C. sinensis* was associated with the presence of a few cholangiocarcinomas in cats and one in a dog. Two experiments in rats were inadequate for evaluation. Infection with *C. sinensis* increased the incidence of cholangiocarcinomas in hamsters treated with 2-acetylaminofluorene or *N*-nitrosodimethylamine.

5.4 Other relevant data

The general pathological features of infection with liver flukes are similar in humans and animals. The changes are characterized by oedema, desquamation and acute inflammatory cellular responses in the bile ducts in the early stage; in the chronic stage, the bile ducts show marked goblet-cell metaplasia, adenomatous hyperplasia and thickening of the walls. Complications may include calculi, suppurative cholangitis and biliary abscess caused by bile stagnation due to mechanical obstruction.

Cholangiocarcinomas appear to arise from pre-existing adenomatous changes in the bile ducts through the phase of intestinal metaplasia or dysplastic change.

The expression of CYP2A isozymes that catalyse the metabolism of aflatoxin and nitrosamines in the liver is increased in *O. viverrini*-infected hamsters. The increased expression is located in regions of the liver adjacent to the site of inflammation. The activity of macrophage-associated nitric oxide synthase is also increased in these animals. No information was available about the effects of liver fluke infection on carcinogen metabolism in humans. Increased urinary levels of nitrate and certain nitrosamines are detected in people infected with *O. viverrini*.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in humans for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in humans for the carcinogenicity of infection with *Clonorchis sinensis*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Clonorchis sinensis*.

In making the overall evaluation, the Working Group noted that experimental and epidemiological studies on *Clonorchis sinensis* confirm:

(i) that the biological and pathological characteristics of *Opisthorchis* and *Clonorchis* are similar;

(ii) that cholangiocarcinoma occurs in infected animals, especially when infection is combined with administration of known carcinogens; and

(iii) that the relative risks for cholangiocarcinoma, and not for hepatocellular carcinoma, are consistently increased in people infected with this organism.

Overall evaluations

Infection with *Opisthorchis viverrini* is carcinogenic to humans *(Group 1)*.

Infection with *Opisthorchis felineus* is not classifiable as to its carcinogenicity to humans (Group 3).

Infection with *Clonorchis sinensis* is probably carcinogenic to humans (Group 2A).